CONSERVATION BIOLOGY APPLIED TO FISH: THE EXAMPLE OF A PROJECT FOR REHABILITATING THE MARBLE TROUT (*SALMO MARMORATUS*) IN SLOVENIA

by

Alain CRIVELLI (1), Gilles POIZAT (1), Patrick BERREBI (2), Dusan JESENSEK (3) & Jean-François RUBIN (4)

ABSTRACT. - The conservation status of freshwater fish is of concern throughout the world, a third of all known species having become extinct or being endangered. Many of them have fragmented populations of small size whose future is in doubt. Conservation biology is the discipline that is used to study such populations, so that they can be managed in a way that will ensure their long-term survival. The history, development and future of conservation biology are described. Following repeated restocking of brown trout (*Salmo trutta*) dating from 1906, the marble trout (*Salmo marmoratus*) seems to have disappeared as a result of genetic pollution (hybridisation) from the lower reaches of the Soca River in Slovenia. A project for rehabilitating the marble trout has been undertaken and will serve as an example to illustrate an application of conservation biology to freshwater fish. A preliminary exploratory stage (1993-1995) that resulted in the publication in 1996 of an Action Plan, written in English and Slovenian, suggested that the cause of the disappearance of the marble trout was hybridisation, established the validity of the project in the region in question and provided us with essential information (occurrence of genetically pure populations) that could be used to define our future strategy. We chose genetic rehabilitation, i.e., the replacement of a population of introduced and introgressed fish by a population of genetically pure fish of the native species, rather than a programme of eradicating undesirable fish by chemical methods, which would have been incompatible with the region's context. Our overall strategy therefore had two main aims: to ensure the long-term survival of populations of pure marble trout (species conservation) and to rehabilitate the genes of marble trout in the hybridisation zone until foreign genes have almost been eliminated.

RÉSUMÉ. - La biologie de la conservation appliquée aux poissons: l'exemple du projet de la réhabilitation de la truite marbrée (*Salmo marmoratus*) en Slovénie.

La situation des poissons d'eau douce dans le monde est préoccupante, un tiers des espèces connues auraient disparu ou seraient fortement menacées. Beaucoup d'entre elles ont des populations fragmentées de petite taille dont le devenir est problématique. La biologie de la conservation est justement la discipline qui s'appliquera à étudier ces populations afin de les gérer de telle façon que leur pérennité soit assurée. L'historique, le développement et l'avenir de la biologie de la conservation sont décrits. Suite à des repeuplements répétés dès 1906 de *Salmo trutta fario*, la truite marbrée, *Salmo marmoratus* aurait disparu par pollution génétique (hybridation) du bassin amont de la rivière Soca en Slovénie. Un projet de réhabilitation de la truite marbrée a été entrepris et nous servira d'exemple pour illustrer une application de la biologie de la conservation aux poissons d'eau douce. Une première phase exploratoire (1993-1995), matérialisée par la publication en 1996 d'un Plan d'Action en anglais

(1) Station biologique de la Tour du Valat, Le Sambuc, 13200 Arles, FRANCE. [a.crivelli@tour-du-valat.com]

(2) Université Montpellier 2, Laboratoire Génome et Populations, CC063.
Present address: ERS 2011 " Ecosystèmes lagunaires", Université Montpellier 2, CP 093, Place E. Bataillon, 34095 Montpellier Cedex 05, FRANCE.

(3) Anglers' Society of Tolmin, Modrej 26c, 65216 Most na Soci, SLOVENIA

(4) Université of Lausanne, Institut d'Écologie, 1000 Dorigny, SUISSE.

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et en slovène nous a permis d'une part d'émettre l'hypothèse que la cause de la disparition de la truite marbrée est bien l'hybridation et d'autre part d'implanter le projet dans la région concernée et de nous donner des éléments essentiels (présence de populations génétiquement pures) pour définir notre stratégie future. Nous avons choisi la réhabilitation génétique, c'est-à-dire le remplacement d'une population de poissons introduits et introgressés par une population de poissons génétiquement purs de l'espèce autochtone, plutôt que de nous lancer dans un programme d'éradication (traitement chimique) des poissons non désirés, incompatible dans le contexte de cette région. Notre stratégie a donc globalement deux grands objectifs: pérenniser les populations de truites marbrées pures (conservation de l'espèce) et réhabiliter les gènes de truites marbrées dans la zone « hybride » jusqu'à une quasi-disparition des gènes étrangers.

Key-words. - Salmonidae - *Salmo marmoratus* - Slovenia - Strategy - Conservation - Hybridisation - Rehabilitation.

The conservation status of freshwater fish is of concern throughout the world, a third of all known species having become extinct or being severely endangered (Pollard *et al.*, 1990; Moyle and Leidy, 1992; Warren and Burr, 1994; Crivelli and Maitland, 1995; Crivelli, 1996; Kirchhofer and Hefti, 1996; Cambray and Bianco, 1998; Leidy and Moyle, 1998; Jonsson *et al.*, 1999; Ricciardi and Rasmussen, 1999). Most of them have fragmented populations of small size whose future is uncertain. Conservation biology is the discipline that is used to study the causes for the decline in such populations and to assess their viability, so that they can be managed in a way that will ensure their long-term survival.

CONSERVATION BIOLOGY

Conservation biology was developed at the end of the 1970s in the United States. It was at this time that conservationists became aware of the increasing extinction of species, of the concept of biodiversity and of the problem of human overpopulation. There was also a feeling that the methods being used by ecologists and land managers to deal with conservation problems were not producing effective results.

In 1980 Soule and Wilcox published the proceedings of a conference with the title "Conservation biology, an evolutionary-ecological perspective", which is generally considered to be the birth of conservation biology (Jacobson, 1990; Caughley, 1994; Caughley and Gunn, 1996; Hunter, 1996; Jacobson and McDuff, 1998; Noss, 1999). The Society for Conservation Biology was founded in 1985, followed by the first publication in 1987 of the journal "Conservation Biology".

This is essentially a multidisciplinary discipline (Jacobson, 1990; Sutherland, 1998; Caro, 1998; Dimmick *et al.*, 1999; Brown, 2000; Poiani *et al.*, 2000), involving not only the life sciences (zoology, botany, ecology), but also physical sciences (geography, geology, chemistry) and also social sciences (socio-economics, law, etc.) and land management (agriculture, forestry, fisheries, etc.). In scientific terms the contribution of island biogeography theory (MacArthur and Wilson, 1967) to the dawn of conservation biology cannot be overlooked (Simberloff, 1997). This is a theory with testable hypotheses, defining the concepts of isolation and of fragmented populations that are nowadays so important. In philosophical terms, the thinking of Aldo Léopold (1949) on the ethics of conservation and on the urgent need to bring together scientists, land man-

agers and conservationists if conservation is to be more effective, were important for structuring the basis of conservation biology.

Very quickly, conservation biology became distinguished from other disciplines and particularly from wildlife biology. Conservation biology differs from wildlife biology mainly on three points: 1) it generates theories and theoretical models which can be applied to practical situations; 2) it assigns the same values to commercial and non-commercial species, and 3) it includes non-biological disciplines such as economics and social aspects. Conservation biology attempts to provide the bases needed for rational long-term management of ecosystems and their resources while maintaining the processes of evolution.

Its philosophy is based on three major principles: 1) evolution is the basic concept of all biology; 2) ecological systems are dynamic and in constant disequilibrium and 3) humans are actively involved in all conservation actions. The first principle implies that the capacity of populations to adapt should be conserved rather than maintaining them in their present state, so that they can respond to future changes. Secondly, ecological systems are essentially dynamic and "climax" or "equilibrium" states are only short-term temporal conditions included in a temporal and spatial dynamic state at a wider scale, hence the constant state of disequilibrium of ecological systems. No protected area is ever completely isolated and disconnected from human influences. Ways must therefore be found of integrating man in all conservation actions.

Associated with these principles, conservation biology is based on two fundamental paradigms according to Caughley (1994): 1) the paradigm of small populations which are interesting because of the effect of restricted population size on survival and 2) the paradigm of declining populations which interests itself in the causes of restricted population size and how to remedy them.

The first paradigm deals with the risk of extinction, applies to all species and is the subject of stochastic theories, whereas the second has no theoretical interest but is interested in the processes leading to species extinction. The weakness of the first is that it does not deal with the causes leading to populations becoming small. The weakness of the second is theoretical, it only applies to individual cases, cannot be generalised and is determinist (e.g., a species does not survive in the most favourable habitat but in the one that is least favourable for the factors leading to decline). For this reason, many people think that the two paradigms cannot be dissociated from one another if you want to be effective (Caughley, 1994; Hedrick *et al.*, 1996).

The first paradigm

The dynamics of a small population and its rate of increase depend both on environment fluctuations and on demographic fluctuations, both of them stochastic phenomena. The latter is constantly varying (changes in recruitment, mortality, etc.) whereas the former amplifies the rate of population increase depending on its frequency and intensity.

The genetic diversity of these small populations could also play a role in the chances of survival. Genetic variability is usually estimated from the mean heterozygosity (= proportion of heterozygous genotypes). Heterozygosity varies greatly between taxonomic groups (0 to 30%) and depending on which marker is used to measure it (Hedrick, 1999). The mean genetic variability, measured on allozymes in 73 freshwater and marine fish species was 0.052% (Mitton and Lewis, 1989; Fig. 1), was 0.022% in 9 populations of genetically pure marble trout and 0.069% in 24 populations of hybrid trout in the Po catchment, Italy (Giuffra *et al.*, 1996) and in the Soca catchment, Slovenia

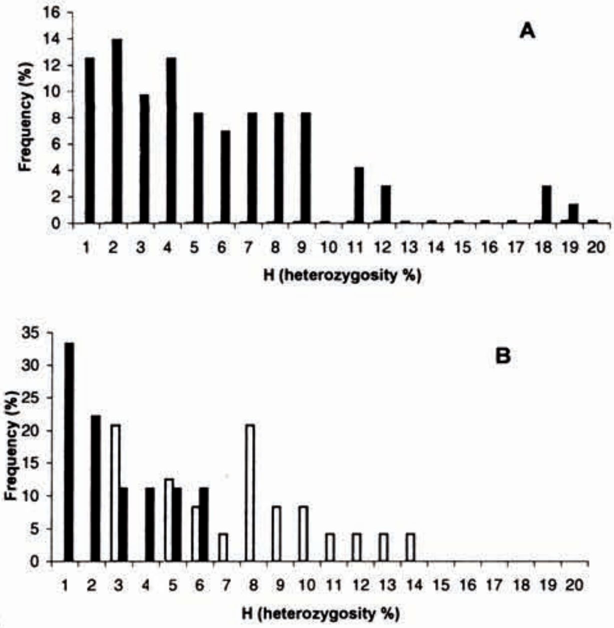

Fig. 1. - Proportion of heterozygosity in 33 populations of fish, 9 populations of pure *Salmo marmoratus* (black bars) and 24 populations of hybrid trout (open bars) in the watersheds of the Po, Italy, and the Soca, Slovenia. **A**: from Mitton and Lewis, 1989; **B**: from Giuffra *et al.*, 1996; Berrebi *et al.*, in press.

(Berrebi *et al.*, in press). A lower genetic variability was recorded in populations of pure marble trout than in the populations of hybrid trout or in fish in general. In *Salvelinus confluentus* (Suckley, 1859), a strongly declining salmonid from the north-western United States, the mean genetic variability of 19 populations was 0.009%, i.e., an extremely low value (Leary *et al.*, 1993), whereas that of 19 species of fish endangered with extinction from the western United States (Echelle, 1991) was 0.034% (varying from of 0 to 0.093%). In theory the higher the value, the greater the adaptive capacity of the organism. However, a low value does not mean that there is a problem or that the population is in danger (Amos and Harwood, 1998). Similarly, a low value of heterozygosity does not necessarily signify a low selective value for a population's life history traits (Allendorf and Leary, 1986; Mitton and Lewis, 1989; Hutchings and Ferguson, 1992; Ferguson *et al.*, 1995; Brito and Coelho, 1997; Sheffer *et al.*, 1997). A reduction or loss of genetic variability is caused by two main factors, which are both related to population size. These are, the founder effect (a small number of individuals not representative of the parental population founds a new population) and the demographic bottleneck (exceptionally high mortality leaves few individuals to continue the affected population, similar to the founder effect). This lack of genetic diversity is more likely to be the result of the processes that initially endangered the species than its cause.

It should be noted that up to now there is no known case where an extinction has been attributable to genetic causes. Populations that are sufficiently large to be ecologically self-sustaining are therefore probably also sufficiently large to remain viable indefinitely in genetic terms (Lande, 1988).

Another concept that is currently very much in fashion and is included in our first paradigm is the metapopulation concept, which is strongly related to habitat fragmentation (Levins, 1969, 1970; Cooper and Mangel, 1999; Hanski, 1999; Rieman and Dunham, 2000). Some habitat changes result in the fragmentation of populations, which then become a metapopulation. There are three types of metapopulations: firstly, the model of Levins (Levins, 1969, 1970), where a series of local populations originating from source patches colonises other patches; some of the local populations may disappear, but the entire metapopulation is resistant to extinction. Secondly, the model of Boorman and Levitt (1973) in which there is a large permanent central population that is the source for the colonisation of peripheral patches which may disappear, and finally the source and sink model of Pulliam (1988), which is similar to that of Levins (1969, 1970), but where there are few source populations and many sink populations, independently of the size of patches. The validity of these models and their relevance to reality is still debatable (Harrison and Taylor, 1996).

There is therefore a random dispersion between patches in a metapopulation which is responsible for the mixing of individuals and therefore for exchanges of genetic information. The concept of permanent or temporary connectivity is essential for existence of a metapopulation. In aquatic systems, and especially for freshwater fish, this connectivity is generally much more restricted than in other taxonomic groups (insects, birds, mammals) which can more readily cross physical barriers within their home range during dispersal (Schmutz and Jungwirth, 1999; Poiani *et al.*, 2000). The dynamics of these metapopulations therefore consists of an alternation between patch colonisation and extinction. The survival of a metapopulation depends as much on the number of patches that it contains as on the distance between these patches. Extinction is related to problem of population size, which itself is a function of demographic and environmental stochastic factors. The dynamics of extinction is still poorly understood. In contrast, colonisation takes place by dispersion (migratory phenomenon) which although very complex is a process that is better understood.

A new concept of "Evolutionarily Significant Units" (ESUs), that is strongly related to the metapopulation concept, to population genetics and to evolutionary processes and systematics, appeared in the early 1980s in the United States when priorities had to be established, conservation units had to be defined and management had to be undertaken for the Pacific salmon (*Oncorhynchus* spp.) which was the subject of a rehabilitation project under the provisions of the U.S. Endangered Species Act (Waples, 1991). The definition of ESUs by Waples (1991) was: « A population or group of populations, that (1) is substantially reproductively isolated from other conspecific population units, and (2) represents an important component in the evolutionary legacy of the species ». Both genetic and ecological diversity are taken into account in this definition. Later, Moritz (1994a, 199b, 1995) redefined this by applying exclusively genetic criteria; he suggested that there should be a distinction between two types of conservation unit: « Management Units (MUs), representing sets of populations that are currently demographically independent; and Evolutionarily Significant Units (ESUs), which represent historically isolated sets of populations that together encompass the evolutionary diversity of a taxon ». An ESU can include several different MUs, but an MU is not necessarily sufficiently distinct to constitute an ESU. Finally, and also related to this new concept, Dodson *et al.* (1998) created the "Operational Conservation Unit" whose definition was: « The unit of conservation that results from the interplay between biological requirements and socio-economic issues. The biological requirements are largely found

within ESU. The OCU therefore reflects the ESU and its interaction with socio-economic issues. In some cases, sufficient economic resources and desire may exist within society to preserve all ESUs and thus the ESUs become the OCUs. In most cases, however, the OCUs may be larger than individual ESUs, encompassing several ESUs into a single OCU ». These new concepts are still the subject of heated debate (Vogler and DeSalle, 1994; Nielsen, 1995; Pennock and Dimmick, 1997; Riddle *et al.*, 1998; Waples, 1998; Paetkau, 1999; Parker *et al.*, 1999; Bininda-Emonds *et al.*, 2000; Crandall *et al.*, 2000), especially as there is no real consensus on the definitions of ESUs and MUs proposed by Moritz (1994a, 1994b). Although some people stress the genetic aspects of this definition and the absence of fixed standards for distinguishing between different ESUs, most reproach it for being a strictly genetic definition and suggest that the definition of ESUs should be based on both genetic and ecological criteria, depending on their respective availability (Ryder, 1986; Waples, 1991; Crandall *et al.*, 2000). Whatever the definitions and criteria that are adopted, it is nevertheless true that prioritisation is essential in every conservation project (Allendorf *et al.*, 1997) and that the conservation units in which the management and conservation activities are to be conducted have to be chosen (Dodson *et al.*, 1998).

The second paradigm

This aims at identifying the cause(s) for the decline in a population of small size. The three most common causes for population declines and extinctions are: habitat destruction and fragmentation, exploitation (commercial exploitation, hunting, fishing, by-catches), and the impact of introduced species, at least over short time scales, although at geological time scales, climatic changes and catastrophic natural events may be more important. Before undertaking any action, it is important to study the biology and ecology of the declining population. Then an attempt is made to list all the factors liable to contribute to the decline. Next, the relevance of these factors is assessed by comparing areas where the species still occurs with those where it has disappeared. Finally, a hypothesis is put forward that is tested experimentally in order to demonstrate that the factor thought to be responsible really is the cause of the decline and is not simply associated with it.

With the progress in computers and the development of user-friendly software, there is an increasing trend in conservation biology toward the use of computer models to predict the future outcome of populations, the most widely known being "Population Viability Analysis" (PVA; see Shaffer, 1981, 1987; Boyce, 1992; Beissinger and Westphal, 1998) and its derivatives (DSP, SSP, Meta and Space, Lindenmayer *et al.*, 1995; Beissinger and Westphal, 1998; BayVAM, Lee and Rieman, 1997). Hedrick *et al.* (1996) believe that Caughley (1994) constructed a false dichotomy between these two paradigms. They stated: « We think it is more productive and accurate to cast the discussion in terms of an analysis of viability that considers both the generally anthropogenic ultimate causes and the stochastic proximate causes - an inclusive population viability analysis. We consider characteristics of population viability analysis (PVA) that are crucial to this inclusive approach ». Later, in their paper, they wrote: « What PVA models bring to the analysis of wildlife populations is the consideration of stochastic processes, but they do not leave out the deterministic threats of habitat loss and alteration, over-harvest, and the impact of exotics ».

Shepard *et al.* (1997) produced, for example, a simulation of the survival probability of 144 populations of cutthroat trout *Oncorhynchus clarki lewisi*, an endangered North

American salmonid, using the BayVAM model (Bayesian viability assessment procedure). This model takes into account the characteristics of the watershed as well as the demographic variables of the species, and showed that in 100 years, only about twenty of the 144 populations surveyed was likely to survive. These models are often seen as aids when decisions have to be made concerning choices of management or conservation measures for vulnerable or endangered species.

Very quickly these models became the subject of arguments between those favouring their use and those who thought they were used indiscriminately and wrongly, and often instead of proper research in the field on the factors responsible for the decline in the population in question. These polemics are still going on (Aber, 1997; Shepard *et al.*, 1997; Beissinger and Westphal, 1998; Reed *et al.*, 1998; Mann and Plummer, 1999; Brook, 2000; Brook *et al.*, 2000). One of the main reproaches is that no reliance can be given to the results of these models because they require information on several variables that is rarely available. Furthermore, it is often difficult to validate them and they often overlook density-dependent effects. Supporters of the use of models meet these criticisms by stating that this is a false problem, the poor quality of demographic data and/or rough estimates being part of the determinist risks in the viability of populations. They also argue that the results of these models are well accepted by site managers, conservationists and decision-makers. The production of guidelines for conducting PVAs and of quality standards for assessing them is the next challenge for the supporters of the use of models in conservation biology (Mann and Plummer, 1999).

In our current state of knowledge, and irrespective of our opinion of these models, conservation biologists are frequently put into a position where they must give quick, clear and simple answers to problems posed by site managers and decision-makers, whereas the answers are usually complicated, sometimes hypothetical, require time and are by their nature uncertain. This is the challenge facing conservation biologists: to retain their scientific credibility while responding with action to problems posed by society. They must therefore reason in terms of probabilities and understand the nature of this uncertainty, which often leads them to apply the principle of precaution, i.e. , to recommend actions having wide safety margins.

What is the future of conservation biology? Rosy, if we believe Wilson (2000) but only under certain conditions: « If conservation biology is to mature into effective science, pure systematics must be accompanied by a massive growth of natural history ». Others think that restoration biology or ecology is quickly going to take over from conservation biology, particularly because of the phenomena of agricultural set-aside or abandonment which will result in vast areas of land in need of restoration and likely to contribute to the conservation of biodiversity (Dobson *et al.*, 1997; Montalvo *et al.*, 1997). According to Young (2000) there is no doubt that: « In short that the long-term future of conservation biology is restoration biology »; even if his opinion is somewhat tempered in the conclusion of his article: « Conservation biology in the short-term and restoration ecology in the long-term are the complementary activities that will form the basis of our belated (but not hopeless) attempt to salvage the disaster (= the disappearance of biodiversity) ».

Nevertheless it seems to us that the first priority in terms of conserving the biodiversity of ecosystems (Ricciardi and Rasmussen, 1999; Wilson, 2000; Myers *et al.*, 2000) is to put all the human and financial resources into preserving those habitats that are still in good condition, that are little degraded, rich in biodiversity, that have retained all their functions and are a source of evolutionary potential. Such an aim comes more

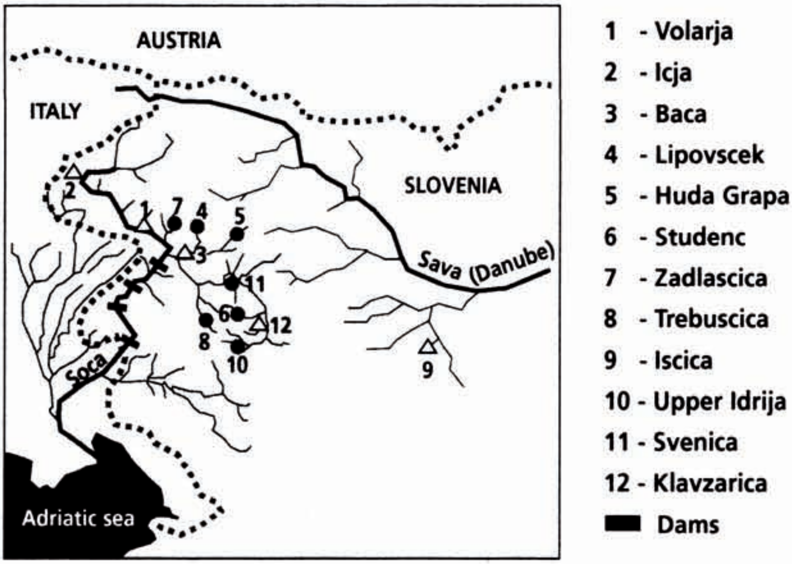

Fig. 2. - Location of hybrid trout and pure *Salmo marmoratus* populations investigated by allozyme analyses (modified from Berrebi *et al.*, in press). Black dots: *S. marmoratus*; open triangles: hybrid trout.

under the heading of conservation biology than that of restoration ecology (Davis, 2000).

Rehabilitation of the marble trout

The rehabilitation project on the marble trout in the upper reaches of the Soca River in Slovenia clearly illustrates what to our mind is a conservation biology project.

Many introduced species or populations of trout have replaced or introgressed with native species of trout, both in Europe and in North America (Campton, 1987; Echelle, 1991; Allendorf and Leary, 1988; Ferguson, 1989; Behnke, 1992; Dowling and Childs, 1992; Hayes *et al.*, 1996; Rhymer and Simberloff, 1996; Poteaux *et al.*, 1998; Aurelle and Berrebi, 1998; Poteaux *et al.*, 1999; Laikre, 1999). In the last twenty years there have been many projects whose aim has been to rehabilitate native trout populations by recommending different strategies, which have sometimes led to much argument (Rinne and Turner, 1991; Meffe, 1992, 1995; Leary *et al.*, 1993; Leary *et al.*, 1995; Krueger and Ihssen, 1995; Gresswell and Liss, 1995; Lange and Smith, 1995; Cummings *et al.*, 1997). These programmes have met with varying success, showing how difficult it is to rehabilitate native species and particularly species that have been subject to hybridisation. In Europe, such rehabilitation activities are still uncommon and very localised.

The marble trout, *Salmo marmoratus* Linnaeus, 1758 or *Salmo trutta marmoratus* (Dorofeyeva *et al.*, 1992; Kottelat, 1997), has a restricted geographical distribution in the basin of the Po in northern Italy (Sommani, 1961; Forneris *et al.*, 1990) and in the Adriatic basin of the former Yugoslavia (Povz, 1995) and Albania (Schöffmann, 1994). It is the largest salmonid in Europe, after the huchen, *Hucho hucho* (Linnaeus, 1758). It can reach a metre in length and weight more than 25 kg. Since the end of the last century, trout *Salmo trutta* of different origins have been introduced into the geographical area

where the marble trout occurs, quickly resulting in hybrid populations (Budihna, 1992; Giuffra *et al.*, 1996). In the Adriatic basin of Slovenia, the first introductions took place from 1906 onward (Povz *et al.*, 1996). Hybrid trout now dominates in most of the rivers in this basin. Before our study started, only one population of marble trout, in the River Zadlascica, was considered on morphological grounds to be pure.

The rehabilitation project of the marble trout was started in 1993 in the upper reaches of the Soca River in Slovenia, all the reaches of the river downstream of the first dam (Fig. 2) being too degraded in terms of hydrology, water pollution and habitat change. Such a project was able to get off the ground in this region for a number of biological and socio-economic reasons. The upper reaches of the Soca River lie in a remarkably well conserved environment (unaltered hydrology, very high water quality, little agricultural and industrial activity, no erosion because of many dense deciduous forests, low human population density and many areas with a protection status, etc.). Many streams and large areas of the upper watershed are officially protected (e.g., Triglav National Park; Povz *et al.*, 1996). Fly-fishing is one of the key and most profitable tourist activities in the region, attracting many anglers from abroad, hence the wholehearted support from local, regional and national authorities for this rehabilitation project. Finally, the anglers' associations, who are legally the managers of the river network are actively involved in the project and particularly the largest of them, the Tolmin Angling Association. Before the project even started, it therefore benefited from considerable advantages that increased its chances of success.

From 1993 to 1996, during phase I of the project, we had seven main objectives: 1) to assess the environmental and socio-economic context of the upper Soca River watershed; 2) to review existing knowledge on the biology and ecology of the marble trout; 3) to determine the genetic diversity of the native, hybrid and introduced trout populations; 4) to look for genetically pure marble trout populations, especially in the headwater streams; 5) to prohibit stocking with trout *Salmo trutta* in the upper reaches of the Soca; 6) to undertake genetic surveys of trout in the hybridisation zone (in the River Volarja) and 7) to conduct an awareness campaign on the project, stressing the long term nature of this project, and its objectives and publishing an Action Plan for the rehabilitation of the marble trout.

The Action Plan was published in 1996 in English for tourists and in Slovenian for local people (Povz *et al.*, 1996). As a result of the first phase of this project, the prohibition on the release of *Salmo trutta* in the Soca watershed became effective in 1996, following the publication of a Slovenian government decree. Since this date, only hybrid trout, known as phenotypic marble trout, have been released into the Soca watershed. Genetic monitoring of the hybridisation zone started in 1993 on the River Volarja, a tributary of the Soca River and was continued in subsequent years with sampling every two years. This tributary was chosen because it was impossible to sample in the Soca River itself because of its size and depth. The population of trout in the River Volarja is considered to be representative of the trout population in the Soca. Preliminary results from the proportions of allozyme alleles showed that the trout population in this zone was composed of one-third *marmoratus* and two-thirds non *marmoratus* (Berrebi *et al.*, in press; Delling *et al.*, in press).

Finally five genetically pure populations (from 0 to 2% foreign genes, Berrebi *et al.*, in press; Fig. 2) with a linear distribution covering more than eight kilometres were discovered in headwater streams, isolated from the hybridisation zone downstream by insurmountable water falls. These populations could therefore provide either breeding

Fig. 3. - A genetically pure marble trout 2+ marked with a Carlin tags from the Gatsnik sanctuary stream (Photograph by Dusan Jesensek).

Table I. - Stream and trout characteristics of the three sanctuary streams. ± SD = ± Standard deviation. IBGN: Indice Biologique Général Normalisé (water quality class: 1 = pristine to 5 = very polluted).

Streams	Gatsnik	Zakojska	Gorska
Stream characteristics			
Length (m)	1,087	845	1,028
Surface area (m^2)	3,516	1,805	3,087
Pool surface (% of total surface)	1,221 (34.7%)	685 (38%)	688 (22.3%)
Altitude range (m a.s.l.)	899-908	400-514	603-728
Number of sectors	6	11	6
Benthos biomass (mg/m^2)	15,938	11,446	14,638
I.B.G.N. (water quality class)	17 (1)	15 (2)	16 (2)
Stream type	two-ways	one-way	one-way
Trout characteristics			
Stocking date	June 1998	June 1996	June 1996
Total number of fish	600	500	500
Density of fish (n/ha)	1,703	1,613	2,754
Mean total length (mm) ± SD	128 ± 16	126 ± 16	115 ± 15
Mean weight (g) ± SD	22 ± 10	22 ± 10	16±8
Mean body condition (K) ± SD	0.99 ± 0.09	1.06 ± 0.12	1.01 ± 0.12

stock or eggs and sperm for a marble trout captive-breeding programme (Gresswell, 1988; Echelle, 1991; Hubert, 1994). Such a programme was started immediately in the fish farms of the Tolmin Angling Association. Although breeding marble trout proved to be more difficult than that of brown trout, it was conducted successfully (Jesensek, 1994).

This first exploratory phase enabled us to hypothesise that the cause of the disappearance of the marble trout was hybridisation. It also installed the project in the region in question, rallied the support of the Idrija Angling Association and provided us with essential information for defining our future strategy. We therefore chose genetic rehabilitation, i.e., the replacement of an introduced, introgressed population of fish by a genetically pure fish population of native species, rather than conducting a programme of eradicating undesirable fish (chemical treatment; Rinne and Turner, 1991), which would have been unthinkable in such an unspoilt region.

Our strategy therefore had two major objectives: to ensure the long-term survival of populations of pure marble trout (species conservation) and rehabilitate the genes of marble trout in the hybridisation zone until almost all foreign (= introduced) genes had been eliminated. This rehabilitation was brought about by repeated restocking with pure marble trout instead of stocking with hybrid trout.

The five pure populations that were identified were clearly insufficient to ensure the survival of the species in the upper watershed of the Soca, so we decided to obtain between 12 and 15 pure populations by the end of the project. This number seems modest in comparison to the 30 populations of Apache trout *Oncorhynchus apache* needed to remove this species from the official American list of species in danger (Dowling and

Table II. - Stream and trout characteristics of the hybridisation experiment. TL = total length (mm) ± SD (± Standard deviation); W = weight (g) ± SD; K = condition factor ($W = aL^3$) ± SD. IBGN: Indice Biologique Général Normalisé (water quality class: 1 = pristine to 5 = very polluted).

Streams	Stopnikarca	Driselpoh	Povrsine Baca	Prodarjeva
Stream characteristics				
Habitat type	1	2	1	1
Surface area (m^2)	523.72	1,433.96	1,350.13	1,181.66
Pool surface (% of the total surface)	129.55 (4%)	284.42 (5%)	290.65 (4.6%)	260.49 (4.5%)
Length (m)	394.6	631.2	517.6	466.9
Altitude range (m a.s.l.)	354.6-300.3	534.5-481.2	625.9-585.6	460.7-413.3
Number of sectors	2	3	3	2
Benthos biomass (mg/m^2)	36,840-39,718	4,710-6,522	779-1,102	3,257-6,686
I.B.G.N. (water quality class)	17 (1)	17 (1)	17 (1)	16 (2)
Trout characteristics				
Total number of fish	156	430	399	300
Density of fish (N/ha)	2,978.8	2,998.7	2,955.3	2,538.8
Brown trout TL	168.49 ± 6.76	146.25 ± 14.73	170.36 ± 32.12	163.14 ± 31.77
Marble trout TL	161.37 ± 24.11	144.73 ± 24.68	153.88 ± 27.44	162.03 ± 29.77
Brown trout W	45.38 ± 5.83	31.29 ± 9.85	53.56 ± 28.96	47.36 ± 27.42
Marble trout W	43.59 ± 26.07	32.14 ± 20.37	40.32 ± 28.26	48.56 ± 32.30
Brown trout K	0.94 ± 0.05	0.97 ± 0.07	0.98 ± 0.08	0.98 ± 0.08
Marble trout K	0.96 ± 0.09	0.96 ± 0.08	0.98 ± 0.07	1.01 ± 0.08

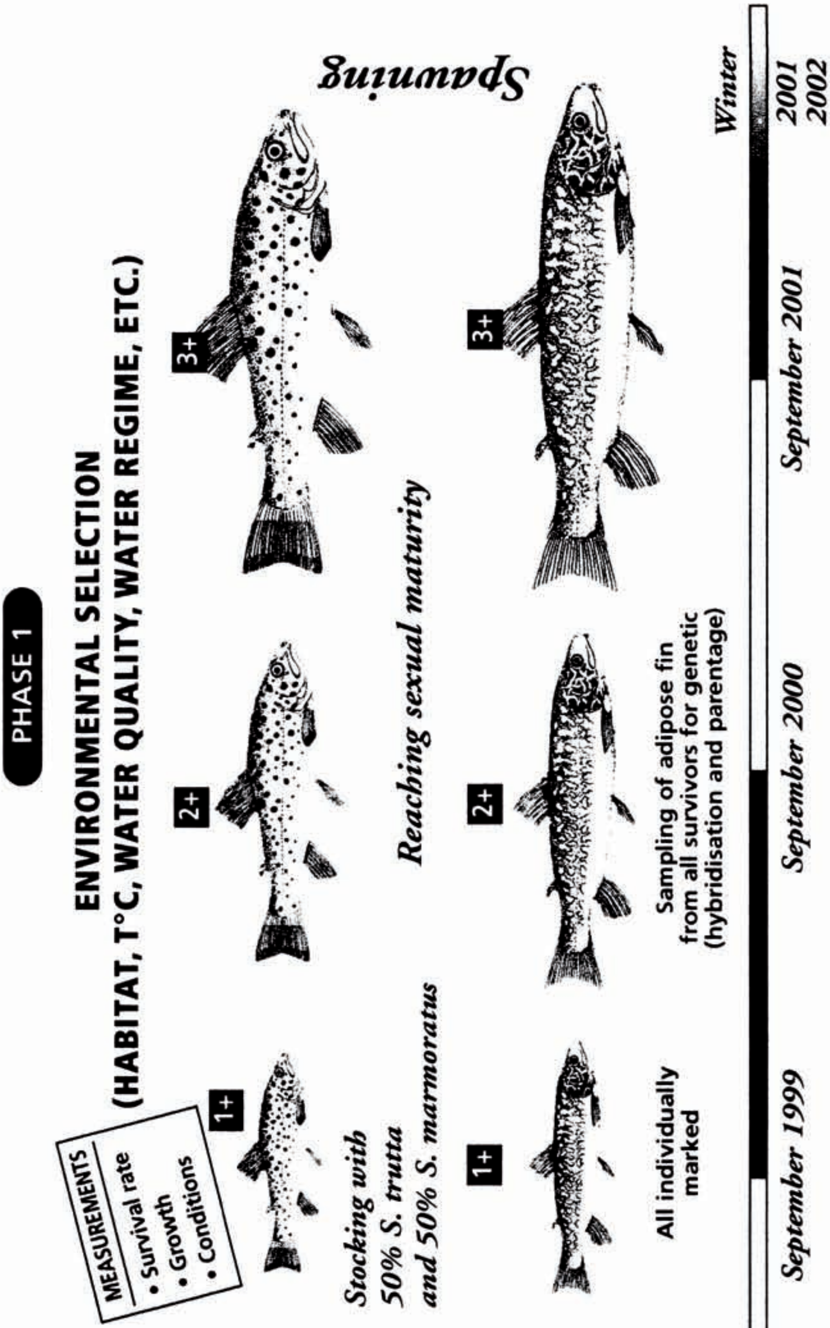

Fig. 4. - Flow chart of the first phase of the hybridisation experiment between *Salmo marmoratus* and *S. trutta* taking place in four streams of the Soca River basin (Slovenia).

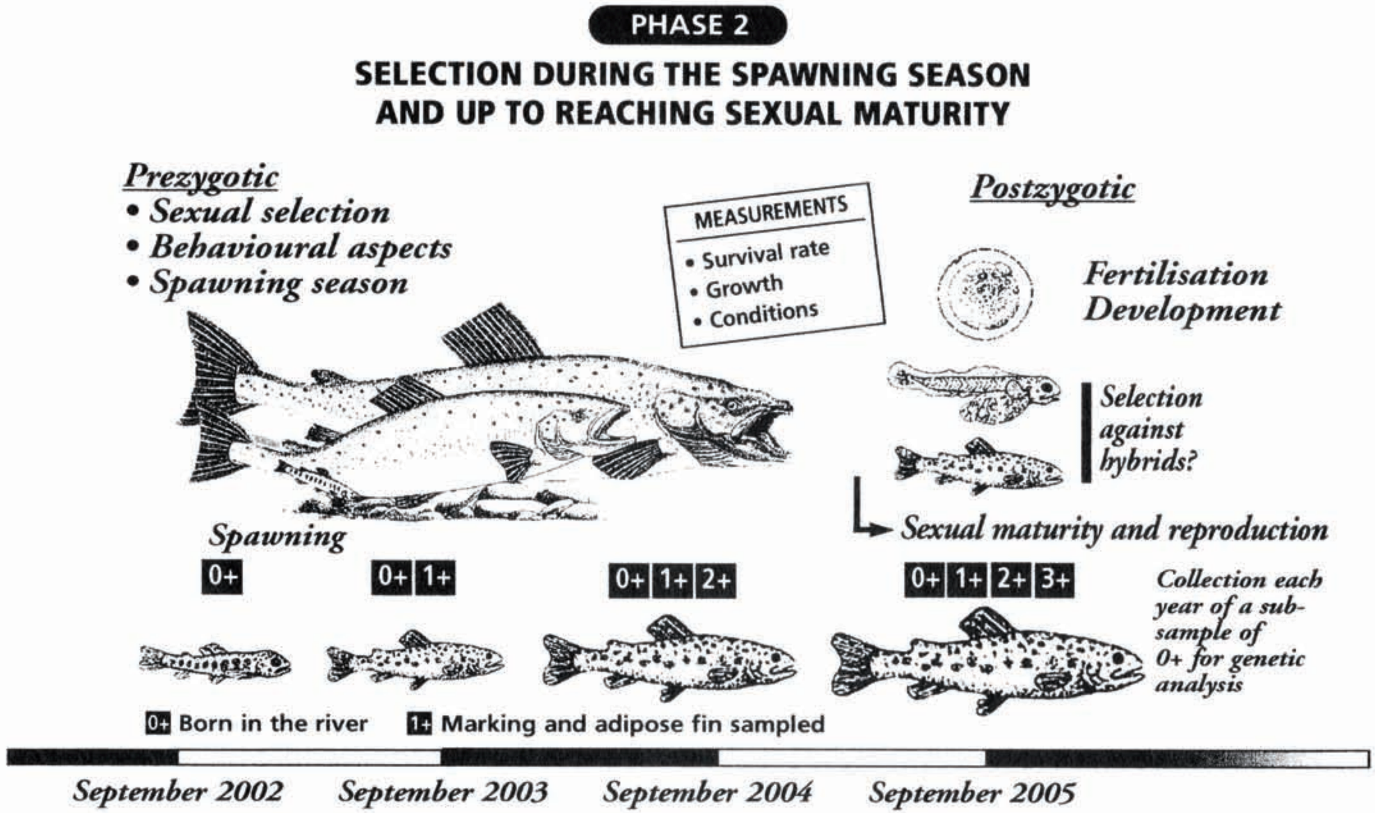

Fig. 5. - Flow chart of the second phase of the hybridisation experiment between *Salmo marmoratus* and *S. trutta* taking place in four streams of the Soca River basin (Slovenia).

Childs, 1992) or compared to the 20 populations of trout defined as one of the objectives of the rehabilitation plan for the trout *Oncorhynchus clarki stomias* (Griffith, 1994). After discussions with our local partners, it seemed that the chances of finding new pure populations was slight (only two new populations have been discovered since 1996; Fig. 2). Consequently it was decided that while still searching for new pure populations in the upper reaches of the Soca River, we would create pure populations in what we would call "sanctuary" streams. Technically this involved finding sections of stream isolated from the hybridisation zone by unsurmountable water falls, that were fish-free and as long as possible so that they could be stocked with genetically pure marble trout bred in a fish farm. This practice has already been attempted in the USA for a number of endangered salmonids (Gerstung, 1988; Marnell, 1988; Propst *et al.*, 1992; Hayes *et al.*, 1996; Spruell *et al.*, 1999). However, in North America, preference is given to transplanting trout collected from genetically pure wild populations to restock fish-free lakes and rivers (Hendrickson and Brooks, 1991; Minckley, 1995), rather than using trout bred in captivity, a more costly process.

Our choice was guided by the small size of the pure populations. Three "sanctuary" streams have been created so far (Table I). The genetically pure marble 1+ trout that were released into these streams were all individually marked with Carlin tags (McGregor and Peake, 1998) and are monitored every year (Fig. 3). Preliminary results are encouraging since good survival and growth have been recorded in the introduced trout and they bred successfully in the Zakojska and Gorska streams in the winter of 1998-1999.

Our hypothesis was that hybridisation was the cause of the disappearance of marble trout in the upper reaches of the Soca River. However, the mechanisms of this hybridisation remain poorly understood. Are the hybrids that occur in the hybridisation zone the result of hybridisation in situ or do they mainly come from restocking with already hybrid fish? Giuffra *et al.* (1996) suggested that there was a partial reproductive isolation between marble trout and brown trout in the Po basin (see also Largiader and Scholl, 1996). This isolation was probably mainly caused by a pre-zygotic barrier during breeding (sexual selection) and adaptation to different habitats. These uncertainties make it difficult to define an effective strategy for the genetic rehabilitation of the hybridisation zone. Although the genetic monitoring (allozymes) conducted in the River Volarja provides some information and trends, it teaches us nothing about the processes involved. For this reason it was decided to conduct a hybridisation experiment in the field to gain a better understanding of the processes involved in hybridisation and attempt to determine at what levels and when interactions occurred between the two species. We therefore released 50% 1+ of *Salmo marmoratus* trout and 50% of *Salmo trutta* trout (coming from the River Aubonne in Switzerland and possessing 87% Atlantic alleles and 13% Mediterranean alleles, this study) into four replicate streams in September 1999. All the trout had been reared in the Tolmin Angling Association fish farm from the egg stage (Table II).

The first level of interactions involves possible differences in habitat selection between the two species (substrate, water quality, flow rates, ecological and altitudinal preferences, etc.) throughout the period between the release of 1+ trout in September 1999 and their first reproduction, probably during the winter of 2001-2002 (Fig. 4). This selection can be measured by the growth rate, the survival rate and the body condition.

Then the first reproduction will be observed to study the next level of interactions (Fig. 5), which then become mainly behavioural (sexual selection, different breeding seasons, migratory movements related to homing phenomena, etc.). This phase involv-

ing pre-zygotic selection covers the entire spawning season. This is the most difficult to measure as it involves direct or video observations on the spawning sites (the fish are marked with external markings of different colours), which should allow us to monitor what is happening on the spawning sites. The location and number of spawning sites could also play a role, as could the seasonal and diurnal timing of the spawning period, which may not be the same for both species. In every September after reproduction, a sample of 0+ fish will be collected to determine the genetic composition of the offspring. The parentage will be analysed to determine which trout were involved in spawning and with what success. The genetic analysis should therefore allow us in hindsight to understand what happened on the spawning sites.

The post-zygotic selection phase, involving mostly genetic, physiological and behavioural processes (differential mortality in eggs and larvae, counter-selection against hybrids and competition between fry for food), starts immediately after spawning. During this phase, environmental selection again plays a role, as with the parental cohort following release, but in this case as from the emergence of the larvae and not from the age of one year. This latter phase lasts up to the first reproduction of the fish born in the river. As these fish will all be marked from the age of one year, and their adipose fins will be collected, we will be able to measure their survival rate, growth and body condition in relation to the genetic status of the trout surviving up to reproduction. If we can continue this project up to 2008, we should have measurements for four cohorts in each of the four replicates. The dynamics and genetics of the populations could then be used to test for any possible pre-zygotic or post-zygotic reproductive isolation between the two trout species over four consecutive years. We intend to use several microsatellites, which would allow us to monitor the genealogy of these small experimental populations over several years.

In the period 1996-2008 we will study and test the two fundamental paradigms of conservation biology. We hope therefore that this project will not only ensure the survival of the marble trout, but will also help us to define the best strategy to adopt for restocking the hybridisation zone and thus rehabilitate the genes of marble trout in whole of the upper Soca watershed.

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